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A paradoxical response of the rat organism to long-term inhalation of silica-containing submicron (predominantly nanoscale) particles of a collected industrial aerosol at realistic exposure levels

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ABSTRACT

While engineered SiO_2 nanoparticle toxicity is being widely investigated, mostly on cell lines or in acute animal experiments, the practical importance of as well as the theoretical interest in industrial condensation aerosols with a high SiO_2 particle content seems to be neglected. That is why, to the best of our knowledge, *long-term inhalation exposure* to nano-SiO_2 has not been undertaken in experimental nanotoxicology studies. To correct this data gap, female white rats were exposed for 3 or 6 months 5 times a week, 4 h a day to an aerosol containing predominantly submicron (nanoscale included) particles of amorphous silica at an exposure concentration of 2.6 ± 0.6 or 10.6 ± 2.1 mg/m^3. This material had been collected from the flue-gas ducts of electric ore smelting furnaces that were producing elemental silicon, subsequently sieved through a < 2 μm screen and redispersed to feed a computerized "nose only" inhalation system. In an auxiliary experiment using a single-shot intratracheal instillation of these particles, it was shown that they induced a pulmonary cell response comparable with that of a highly cytotoxic and fibrogenic quartz powder, namely DQ12. However, in long-term inhalation tests, the aerosol studied proved to be of very low systemic toxicity and negligible pulmonary fibrogenicity. This paradox may be explained by a low SiO_2 retention in the lungs and other organs due to the relatively high solubility of these nanoparticles. nasal penetration of nanoparticles into the brain as well as their genotoxic action were found in the same experiment, results that make one give a cautious overall assessment of this aerosol as an occupational or environmental hazard.

1. Introduction

In our previous experimental studies (Katsnelson et al., 2011, 2012a, 2012b, 2013, 2014, 2015; Privalova et al., 2014a, 2014b; Minigalieva et al., 2015, 2017), we have shown, in good agreement with the data of some other authors (e.g., Fröhlich, 2013; Fröhlich and Salar-Behzadi, 2014), that a single-shot intratracheal instillation and multiple intraperitoneal injections of a range of metal and metal-oxide nanoparticle (NP) species cause toxic effects "in vivo" at all levels, from cellular to systemic and organismic. These effects are considerably greater than the toxic effects of their micrometric chemical counterparts, one of the probable causes of this being the enhanced capability of such NPs to dissolve in biological media. On the other hand, the experiment with rats that were exposed to long-term inhalation of iron oxide (Fe_2O_3) NPs demonstrated that the overbalancing effect of the same solubility "in vivo" was low retention of these NPs in the organism and, thus, low pulmonary fibrogenicity and systemic toxicity (Sutunkova et al., 2016). It was of interest to test the hypothesis whether such paradoxically low harmfulness under inhalation exposure to cytotoxic nano-aerosols could be characteristic of other relatively easily solubilized NPs as well.

As we have repeatedly emphasized (e.g., Katsnelson et al., 2015), special interest in the toxicology of metal-oxide NPs is not due alone to the fact that they are purposefully manufactured (as so-called engi-

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neered nanoparticles) for various technological, scientific, and medical needs. Researchers in the field of nanotoxicology tend to underestimate the importance of the fact that people employed in metallurgical, welding and other industries and those residing in areas contaminated by atmospheric emissions from these facilities, find themselves being exposed chronically to such NPs that constitute a more or less substantial proportion of the submicron fraction of condensation aerosols by-produced in relevant “old” technologies. This renders the issue of metal and metal oxice nanotoxicology much more complex because these aerosols are, as a rule, both polydisperse and multi-component (Minigalieva et al., 2015, 2017).

The above is fully applicable to the condensation aerosols of such a typical *nonmetal* as silicon. These aerosols are released in the course of steel making (particularly when steel is being deoxidized with silicon in the ladle), production of ferrosilicon and other siliceous ferroalloys, arc welding (since all types of electrode coating include liquid glass, i.e. sodium silicate) and, above all, production of elemental silicon also called crystalline or metallic silicon.

This is an ore-thermal process conducted in an electric arc furnace with an open top through which crushed quartzite and coke are charged. The intermediate by-product of crystalline dioxide reduction to elemental silicon in this process is SiO monoxide. While in the furnace, this substance is in the gaseous state, being carried away from it with the outgoing stream of hot gases. But when it mixes with the ambient air being drawn in, it gets cooled and oxidized to silicon dioxide, SiO_2, which then condenses as a polydisperse aerosol consisting of spherical submicron-sized particles including a considerable percentage of nanoparticles (Velichkovsky and Katsnelson, 1964). It is this aerosol that furnace operators are exposed to at a concentration of 11,6 ± 2,2 mg/m^3 (Kulikov, 1995) and that is exhausted through the hood and downstream gas duct into the atmosphere. Similar concentrations of this aerosol at an average of 10,3 mg/m^3 were discovered by Petin (1975) in the workplace air near a furnace at a facility (producing ferrosilicon containing 75% of silicon.

As early as back in the late 1950s–early 1960s, B.T.Velichkovsky showed experimentally that SiO_2-containing condensation aerosols accompanying the production of various siliceous ferroalloys, can induce experimental silicosis, being sometimes even more fibrogenic than quartz dust (Velichkovsky and Katsnelson, 1964). These data were, however, obtained under intratracheal instillation of powders in standard doses (50 mg), i.e. under dust loads known to be excessive on rat lungs.

The only chronic inhalation experiment with such aerosols that lasted 6 months was, to the best of our knowledge, carried out by Petin (1978), who loaded into the dust feeders of 4 chambers operating in parallel dust samples swept off the plates of the electrodes on furnaces smelting ferrosilicon grades Si75, Si45 and Si18 (containing 82,9%, 61,0% and 30,8% of free silicon dioxide, respectively) or quartz dust (89,9% of free silicon dioxide in the form of alpha quartz). This researcher discovered, beyond any doubt, the onset of typical experimental silicosis which under exposure to aerosol with the highest concentration of amorphous silicon dioxide was even more intensive than under exposure to quartz dust. However, whereas two months after the termination of the inhalation exposure to quartz dust the silicotic pulmonary fibrosis continued progressing, the silicosis caused by the condensation aerosol with high SiO2 content demonstrated a reversal. The latter was explained by a much quicker clearance of the pulmonary tissue from SiO_2, which, in turn, could be explained by a greater solubility of the particles. In this case, too, we should emphasize the explicitly aggravated exposure conditions, with an average concentration of the aerosols in all chambers being 200 mg/m^3, i.e. 20 times higher than actual exposure levels.

A question arises whether it could be possible that for relatively low realistic levels of exposure the beneficial role of the solubility would prevail so much over its adverse effect that the ultimate changes in the lungs would prove to be very weak. This is exactly what was also observed by us (Sutunkova et al., 2016) in a chronic inhalation experiment with Fe_2O_3 nanoparticles at a low concentration (about 1 mg/m^3). However, although it is quite likely to be a general pattern, it needs verifying in each particular case.

In the above-mentioned experiment, Petin (1995) did not discover any dependence of the changes in some organs and systems of rats chronically inhaling condensation aerosols or quartz dust on silicon dioxide solubility, suggesting that the latter is as a favorable property which facilitates the elimination of silicon dioxide from the lungs without creating any danger of intoxication. However, this conclusion also needs to be verified both because in that experiment the researcher did not estimate the accumulation of silicon dioxide in various rat organs and blood and because the list of indices used for assessing the status of the organism was rather limited.

With the arrival of the «nanotechnology era», engineered nanoparticles of amorphous silica have become one of the five most widely consumed nano-products (Vance et al., 2015), and therefore compelled the attention of the toxicologists while understanding toxicological importance of inhalation exposures to spontaneously by-produced analogs of these NPs seems to have remained in the past. It is possibly why, given a great number of experiments with engineered SiO_2-NPs that have been carried out and are still conducted «in vitro» on cell cultures or, less often, «in vivo» in acute experiments under parenteral exposure (see, e.g., Park and Park, 2009; Eom and Choi, 2009; Kim et al., 2010; Sergent et al., 2012; Du et al., 2013; Petrick et al., 2016; Guo et al., 2015, 2016; Wang et al., 2017, and a lot of others), we have failed to find information on long-term inhalation exposure studies with this nanomaterial.

Recognising that such exposure is of real practical value in relation not so much to engineered SiO_2-NPs as to SiO_2-NPs component of the above-mentioned industrial aerosols, we set ourselves the task of conducting a chronic inhalation experiment to study the health effects of one of such aerosols in which, based on our earlier experience (Velichkovsky and Katsnelson, 1964), we could expect the presence of a substantial fraction of spontaneously by-produced SiO_2-NPs. Specifically, we have chosen for an inhalation study the submicron fraction of an aerosol emitted from silicon-producing ore-thermal furnaces.

The goal of this study was to not only characterize this particular aerosol but also come closer to verifying the general hypothesis stated previously: whether highly cytotoxic NPs can demonstrate low systemic toxicity and low pulmonary fibrogenicity on realistic inhalation exposure. As an independent test for particle cytotoxicity and acute pulmonotoxicity we used shifts in pulmonary free cell population in response to a single-shot intratracheal instillation of particles under study compared with those of DQ12 quartz. This test was long ago established as a good predictor of pulmonary fibrogenicity under long-term exposure to the micrometric particles (Katsnelson et al., 1994, 1995). However, we shall show that in the case of SiO_2-NPs this predictor can be deceptive due to toxicokinetic effects of these NPs solubilization.

2. Materials and methods

For a solid aerosol generator, we used the Palas RBG 1000 ID dispersing unit charged with a powder obtained by sieving through a < 2 μm screen the dust that had been collected in the horizontal section of the flue gas duct from the hood over an ore-thermal furnace. The scanning electron microscopy of this material revealed quantitative prevalence of particles of a regular spherical shape with a diameter of less than 100 nm (Fig. 1a).

It should be noted that the silicon smelting process in the ore-thermal furnace produces gaseous silicon monoxide, SiO, which gets oxidized and condenses to SiO_2 particles as the gas flow is cooled by the air being drawn along with it. There are no reasons to doubt that the above-mentioned spherical NPs and similar dia. > 100 nm submicron particles represent just the condensation aerosol of silicon dioxide.